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Effects of Soybean Density and Sowing Time on the Yield and the Quality of Mixed Silage in Corn-Soybean Strip Intercropping System

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Abstract: Intercropping is a cropping strategy that makes efficient use of space, nutrients, and soil. A 2-year field trial was conducted in 2019 and 2020 to study the effects of different soybean sowing times (9 days before corn sowing (ST1), 0 days at corn sowing (ST2), and 9 days after corn sowing (ST3), respectively) and densities (120,000 plants ha^{-1} (PD1), 150,000 plants ha^{-1} (PD2) and 180,000 plants ha^{-1} (PD3), respectively, and the planting density of corn was 60,000 plants ha^{-1} constantly) on total yield and on mixed silage quality in corn-soybean strip intercropping system. The yield decreased with an increase in soybean planting density. Before ensiling, the total dry matter (DM) content increased with an increase in soybean planting density, while that of crude protein content decreased with sowing time. The interaction of planting density × sowing time was significant for neutral detergent fiber and water-soluble carbohydrate (WSC) content. After ensiling, the WSC content of PD2ST3 (4.90% DM) was the highest. The PD1 (4.51%) had a higher content of ammonia–nitrogen to total nitrogen than that of PD2 and PD3. The lactic acid content of PD2ST3 (3.14% DM) was the highest. In general, better silage quality and a higher total yield were obtained when soybean was sown at the planting density of 150,000 plants ha^{-1} after 9 days of corn sowing.

Keywords: corn-soybean intercropping; fermentation quality; planting density; silage; sowing time; yield

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1. Introduction

As the world population and the demand for cultivated land continues to increase, the crescent demand for high-producing lands is challenged by the shortage of high-quality food. Intercropping is proven to offer a more efficient use of light, space, water, and soil nutrients that may alleviate the problem of reduced arable land [1,2]. Corn (*Zea mays* L.) is one of the most cultivated crops in the world, and 67% of corn is used as a source of livestock feed globally, such as silage [3]. Moreover, in the case of the same energy content of the feed, the addition of corn silage in dairy rations is a cost-effective feed for dairy cattle that potentially improves feed intake and milk yield [4–6]. Whole-plant soybean (*Glycine max* L. Merr.) is rich in protein (15–20%) and vitamins [7–9]; and, in most cases, it is well-preserved by ensiling when harvested between the growth stages of R5 (seed filling) and R7 (beginning maturity) [10,11]. However, an improper harvest time for ensiling makes it prone to produce a relatively high butyric acid content, which is usually the result of a relatively low water-soluble carbohydrate (WSC) content and a high buffering capacity, causing nutrient losses [12].

Therefore, the combination of the corn and the soybean silage is complementary, and the intercropping of the two crops shall facilitate this silage-making. Corn-soybean intercropping came from the intercropping of corn and potato in the 1970s, as potato gradually became a subsidiary food from a staple food along with the difficulty of storing and transporting potato, while that of corn-soybean intercropping became popular due to greater profitability and convenience [13,14]. Accordingly, corn-soybean intercropping has multiple advantages such as a high resource utilization rate, protein yield, and biomass yield and it has less nitrogen fertilizer input by soybean nitrogen fixation [13,15,16], which improves productivity and environmentally sound sustainability [17,18]. Chen et al. [15] showed that corn-soybean intercropping system can improve nitrogen use efficiency and the land equivalent ratio (1.59–1.61). Therefore, the corn-soybean strip intercropping system has now become a common culture strategy in China.

The traditional corn-soybean intercropping system is a strategy of sowing and harvesting multiple plants synchronously for grain production [19]. As the meat requirement is ever-increasing globally with improvements in living standards [20], the addition of legumes in silage has also increased because it improves animal performance, especially meat production [21]. However, the shortage of quality feed has restricted the development of modern animal husbandry [22]. Ensiling is a whole crop use practice guaranteeing year-round animal feed. If whole crop corn and soybean were harvested and ensiled simultaneously, it would improve the nutritional value of the feed and increase the resource utilization rate [23].

An improper management of sowing time may lead to interspecific competition impacting the growth and the development of crops [24], and the planting density of soybean also directly affects its growth and silage quality [25]. Yong et al. [26] found that the early sowing and the proper high planting density of corn increased the corn–soybean total yield, while Cardoso et al. [27] and Prasad and Brook [28] reported that the increase in soybean density decreased the total yield. Zeng et al. [9] reported that corn–soybean mixed silage improved nutrition content and fermentation quality versus corn/soybean sole silage. However, there is a shortage of reporting on how the sowing time and the planting density of soybean jointly affect the yield and the silage quality in a corn–soybean strip intercropping system. Meanwhile, most studies about corn-soybean intercropping focus on grain production, and the interval of soybean sowing time is usually approximately 20 days as grain harvest does not require much moisture for the plant [29,30]. However, successful ensiling requires appropriate moisture (60–70%), thus the current study shortened the sowing time interval to 9 days to avoid over water losses of the soybean harvest caused by high maturity [31].

Therefore, this study aimed to obtain the optimal sowing times and densities of soybean for quality mixed silage and a high total yield in a strip intercropping system on the condition of constant corn planting density by adjusting the plant spacing and strip width among different soybean planting densities.

2. Materials and Methods

2.1. Experimental Site

A 2-year field experiment was conducted in 2019 and 2020 at the Chongzhou base of the Sichuan Agricultural University (30°33′ N, 103°38′ E), which had an annual mean rainfall of 969 mm and a temperature of 16.08 °C. The weather data of the cropping period is shown in Table 1. The type of soil is Entisols, and the basic information of the soil (in the top soil layer (0–20 cm)) is shown in Table 2 [32,33].

2.2. Field Experiment and Silage Preparation

The corn cultivar was Yayu 04889 (Sichuan Yayu Technology Development Co., Ltd., Ya'an, China; growth period: 98 days), and the soybean cultivar was Nandou 35 (Nanchong Academy of Agricultural Sciences, Nanchong, China; growth period: 120.6 days). The experiment was laid out in a split-plot design and each treatment had three replicates. All replicates were within a 1.2 m distance of each other to eliminate disturbance from the nutrient flow. Corn and soybean were cultivated by manual hole sowing. The planting density of corn was 60,000 plants ha^{-1} constantly and the sowing time was 5 May in both years. The main plots were the planting densities of soybean (PD1: 120,000 plants ha^{-1}; PD2: 150,000 plants ha^{-1}; PD3: 180,000 plants ha^{-1}), and the subplots were the sowing times of soybean (ST1: 9 d before corn sowing (26 April); ST2: 0 d at corn sowing (5 May); ST3: 9 d after corn sowing (14 May). Considering the intraspecific, interspecies competition, and the identity of the corn planting density in the different corn–soybean intercropping systems [34], the soybean and the corn space arrangements are shown in Figure 1. The area of each experimental plot in PD1 (four strips of corn and four strips of soybean), PD2 (four strips of corn and six strips of soybean), and PD3 (four strips of corn and eight strips of soybean) was 13.2 m^2, 14.4 m^2, and 15.6 m^2.

Table 1. Monthly minimum temperature, maximum temperature, mean temperature, and rainfall from April to August during the cropping period 2019–2020.

Month	Years							
	2019				2020			
	Minimum T (°C)	Maximum T (°C)	Mean T (°C)	Rainfall (mm)	Minimum T (°C)	Maximum T (°C)	Mean T (°C)	Rainfall (mm)
April	15.0	27.7	19.8	44.6	11.7	22.5	15.8	24.1
May	16.9	26.5	20.3	146.3	19.9	29	22.1	62.4
June	20.7	30.1	24.5	6.6	21.3	32.1	25.2	47.5
July	21.6	31.2	24.8	294.3	21.8	31.4	25.3	141
August	22.2	32.1	25.4	32	21.6	30.6	24.8	439.2

Table 2. Basic information of the soil in the experimental area (in the top soil layer (0–20 cm)).

Soil Type	Soil Reaction (pH)	Organic Matter (k kg^{-1})	Available Plant Nutrient Content (mg kg^{-1})		
			N	P	K
Entisols	6.6	30.34	63.5	40.57	96.36

The rate of basal fertilizers was 135 kg ha^{-1} with N, 40 kg ha^{-1} with P and 10 kg ha^{-1} with K in corn rows when corn was sown, respectively. The rate of basal fertilizers was 75 kg ha^{-1} with N, 40 kg ha^{-1} with P and 4 kg ha^{-1} with K in soybean rows when soybean was sown, respectively. Furthermore, 75 kg ha^{-1} with N was applied in corn rows at the V6 (sixth leaf stage) of corn [19]. Irrigation was applied before the sowing of both corn and soybean. Weeds were controlled by hand hoeing. All field management was based on the farmer's practice.

The corn and the soybean were manually harvested on 8 August in 2019 and in 2020 for yield determination (the stubble height of harvesting was 15 cm for corn and 5 cm for soybean), at which time the corn was at 2/3 the milk line stage (approximately 30% dry matter content), and the soybean ST1 was at R8 (full maturity stage), ST2 was at R7 (beginning maturity stage), and ST3 was at R6 (full seed stage). According to the ratio of corn and soybean density: 8 corn plants (4 plants for each row) and 16 soybean plants (8 plants for each row) were taken randomly for treatment PD1; 6 corn plants (3 plants for each row) and 15 soybean plants (5 plants for each row) were taken randomly for treatment PD2; and 8 corn plants (4 plants for each row) and 24 soybean plants (6 plants for each row) were taken randomly for treatment PD3, respectively. Samples were dried for 1 h at 105 °C and then at 65 °C until the weight became constant. Then, the dry matter weight of the whole plant and the yield per unit area were measured and calculated according to the planting density.

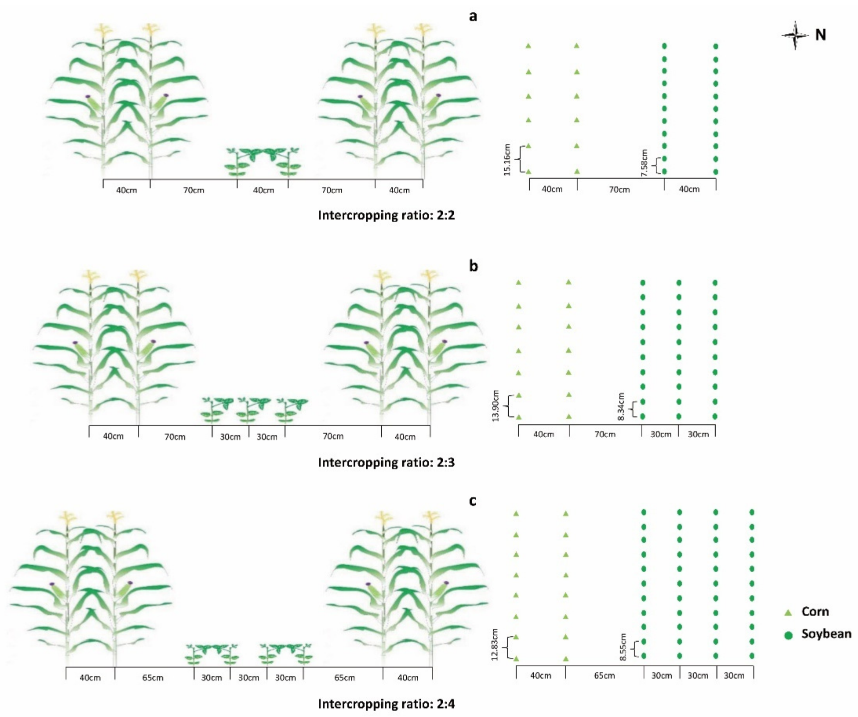

Figure 1. The soybean and the corn space arrangements. (**a**–**c**) represent the layout of intercropping ratio was 2:2 (60,000 plants ha^{-1} for corn, 120,000 plants ha^{-1} for soybean), intercropping ratio was 2:3 (60,000 plants ha^{-1} for corn, 150,000 plants ha^{-1} for soybean), and intercropping ratio was 2:4 (60,000 plants ha^{-1} for corn, 180,000 plants ha^{-1} for soybean), respectively.

Silage treatment was conducted corresponding to the three different plots in the field with the same sampling method with yield determination in 2020. A total of 27 bags were used (3 planting densities × 3 sowing times × 3 replicates). The corn and the soybean were processed into 20 mm by smasher (3-Phase induction motor, China) and divided into two parts: one for determination of the chemical composition of the fresh samples before sealing and the other packed into vacuum-sealed polyethylene plastic bags (30 cm × 40 cm, China), which were vacuum sealed with a vacuum sealer (evox-30, Orved, Italy) and kept at room temperature (25–28 °C). Each plastic bag was filled with 0.5 kg of fresh matter [9]. The chemical composition and the fermentation profile were determined after 60 d of storage.

2.3. Chemical Composition Analysis

The fresh samples and silage samples were dried to a constant weight in a forced-air oven at 65 °C for 72 h to determine dry matter (DM) content. Dried samples were ground to pass through a 1-mm Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) screen for chemical analysis. The water-soluble carbohydrate (WSC) content was determined according to anthranone colorimetry [35], and the crude protein (CP) content was determined by the Kjeldahl method [35]. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were analyzed (sodium sulfide and α-amylase were used) by the Ankom system, using the special filter bag according to Van Soest et al. [36].

2.4. Fermentation Profile Analysis

According to Zeng et al. [9], 20 g of silage samples were homogenized with 180 mL of deionized water and then sealed, after that it was filtered with 4 layers of gauze, and the water extract was immediately used for determination of pH. Part of the water extract was used for determining the ammonia–nitrogen content to total nitrogen (NH_3-N/TN) and lactic acid (LA), acetic acid (AA), propionic acid (PA), and butyric acid (BA). The pH value of the extract was measured using a portable pH meter (PHSJ-5; Leici, Shanghai, China). The content of ammonia–nitrogen was determined by phenol sodium hypochlorite colorimetry [37]. Portions of the water extract were subjected to the concentrations of LA, AA, PA, and BA determination after being centrifuged at 12,000× *g* at 4 °C for 10 min and passed through a 0.22 mm membrane via high-performance liquid chromatography (HPLC, kc-811, shimadzu Co., Kyoto, Japan), which used an HPLC adopted ultraviolet detector with a detection wavelength of 210 nm, a mobile phase of 3 mmol L^{-1} perchloric acid (0.5 mL min^{-1}), and a temperature of 55 °C [31].

2.5. Statistical Analyses

The data of each treatment were compared and analyzed by means of average value. The differences between means were assessed by the method of LSD comparison ($p < 0.05$). All data were displayed using the analysis of variance (IBM SPSS 19.0, Chicago, IL, USA).

3. Results

3.1. Dry Matter Yield

As shown in Table 3, corn, soybean, and the total yield were all affected by soybean density and they showed a similar trend in both years ($p < 0.01$), with the highest values found in PD1. With the increase in soybean planting density, the corn dry matter yield, soybean dry matter yield, and the total dry matter yield decreased. In 2019, the corn dry matter yield of PD1 was higher than that of PD2 and PD3 by 15.03% and 21.29%, respectively; the soybean dry matter yield of PD1 was higher than that of PD2 and PD3 by 16.72% and 19.77%, respectively; the total dry matter yield of PD1 was higher than that of PD2 and PD3 by 15.57% and 20.80%, respectively. In 2020, the corn dry matter yield of PD1 was higher than that of PD2 and PD3 by 15.72% and 22.31%, respectively; the soybean dry matter yield of PD1 was higher than that of PD2 and PD3 by 18.75% and 22.13%, respectively; the total dry matter yield of PD1 was higher than that of PD2 and PD3 by 16.61% and 22.25%, respectively.

3.2. Chemical Composition of Fresh Samples

Different soybean planting densities and sowing times affected the chemical composition of corn-soybean intercropping (Table 4). The DM content was affected by soybean density ($p < 0.05$), and it increased with the increase in soybean planting density. Moreover, the DM content of PD2 and PD3 was 1.7–2.29% higher than that of PD1. The CP content decreased with the delay in soybean sowing time ($p < 0.01$), among which ST1 was 0.70% higher than ST3 ($p < 0.01$), and no significant differences were found between ST2 and the others. The interaction of planting density × sowing time was significant for NDF and WSC content ($p < 0.01$); the NDF content was also affected by sowing time and planting density ($p < 0.01$). At the same planting density, the NDF content of ST1 was higher than that of others, among which PD1ST1 was the highest (56.27% of DM). Except for PD3, the content of NDF decreased when the sowing time was prolonged. At the same sowing time, the NDF content of PD2 was lower than that of the others. The WSC content was affected by sowing time, planting density, and their interactions ($p < 0.01$), and the WSC content of ST3, ST1, and ST2 was the highest for PD1 (13.31% of DM), PD2 (14.63% of DM) and PD3 (9.66% of DM), respectively. The WSC content of PD2ST1 (14.63% of DM) and PD1ST3 (13.31% of DM) was the highest.

3.3. Chemical Composition of Corn and Soybean Mixed Silage

After 60 days of ensiling, different soybean planting densities and sowing times affected the chemical composition of the corn and the soybean after mixed silage (Table 5). The interaction of planting density and sowing time only affected the WSC content ($p < 0.01$), and the WSC content was also affected by the soybean density ($p < 0.01$). The WSC content of PD2ST2 and PD2ST3 was the highest (4.34 and 4.90% of DM). The DM and the CP were affected by soybean density ($p < 0.05$). The DM content of PD3 was higher than PD1, while no differences were observed in comparison to PD2 ($p < 0.05$). The lowest CP content was found in PD1, which was lower than those of PD2 and PD3 ($p < 0.05$).

Table 3. Effects of sowing time and planting density of soybean on dry-matter yield (kg ha^{-1}) in corn-soybean strip intercropping system.

Item	Corn Yield (kg ha^{-1})				Soybean Yield (kg ha^{-1})				The Total Yield (kg ha^{-1})			
	ST1	ST2	ST3	Mean	ST1	ST2	ST3	Mean	ST1	ST2	ST3	Mean
						2019						
PD1	25,254	25,906	25,310	25,490 ^{a}	10,246	11,044	12,909	11,400 ^{a}	35,498	36,956	38,212	36,889 ^{a}
PD2	22,138	21,706	22,633	22,159 ^{b}	9350	10,289	9663	9767 ^{ab}	31,480	32,001	32,274	31,918 ^{b}
PD3	20,251	20,436	22,360	21,016 ^{b}	8889	10,217	9449	9518 ^{b}	29,152	30,655	31,804	30,537 ^{b}
Mean	22,548 ^{a}	22,682 ^{a}	23,434 ^{a}		9495 ^{b}	10,517 ^{a}	10,674 ^{a}		32,043 ^{a}	33,204 ^{a}	34,097 ^{a}	
SEM		475				272				634		
Planting density (PD)		<0.001				0.002				<0.001		
sowing time (ST)		0.497				0.046				0.082		
PD × ST		0.706				0.144				0.894		
						2020						
PD1	24,251	24,908	24,307	24,488 ^{a}	9247	10,048	11,900	10,398 ^{a}	33,497	34,955	36,207	34,886 ^{a}
PD2	21,137	20,714	21,636	21,162 ^{b}	8344	9287	8637	8756 ^{ab}	29,480	30,001	30,273	29,918 ^{b}
PD3	19,260	19,444	21,363	20,022 ^{b}	7890	9210	8441	8514 ^{b}	27,151	28,654	29,804	28,536 ^{b}
Mean	21,549 ^{a}	21,688 ^{a}	22,435 ^{a}		8494 ^{a}	9515 ^{a}	9659 ^{a}		30,043 ^{a}	31,203 ^{a}	32,095 ^{a}	
SEM		475				273				636		
p value												
Planting density (PD)		<0.001				0.002				<0.001		
sowing time (ST)		0.571				0.522				0.790		
PD × ST		0.710				0.150				0.891		

Data are the mean and the standard error of the mean of the 3 samples. For items with significant interaction effects of PD × ST, significance analysis was conducted among all different treatments. For items with insignificant interaction effects of PD × ST, significance analysis was conducted between the mean values of major factors and secondary factors. Lower case letters with different superscripts indicate the differences between different treatments ($p < 0.05$). SEM, standard error of the mean; PD1, the planting density of soybean was 120,000 plants ha^{-1}; PD2, the planting density of soybean was 150,000 plants ha^{-1}; PD3, the planting density of soybean was 180,000 plants ha^{-1}; ST1, the sowing time of soybean was 9 d before corn sowing; ST2, the sowing time of soybean was 0 d before corn sowing; ST3, the sowing time of soybean was 9 d after sowing corn.

Table 4. Effects of sowing time and planting density of soybean on chemical composition of fresh samples in corn-soybean strip intercropping system.

Item	DM (%)				CP (% of DM)				NDF (% of DM)				ADF (% of DM)				WSC (% of DM)			
	ST1	ST2	ST3	Mean	ST1	ST2	ST3	Mean	ST1	ST2	ST3	Mean	ST1	ST2	ST3	Mean	ST1	ST2	ST3	Mean
PD1	36.68	36.50	36.43	36.54 ^{b}	8.88	8.75	8.40	8.68 ^{a}	56.27 ^{a}	53.67 ^{d}	53.33 ^{de}	54.42	30.93	31.13	31.43	31.16 ^{a}	8.53 ^{cd}	8.60 ^{cd}	13.31 ^{a}	10.15
PD2	37.95	38.54	38.24	38.24 ^{a}	8.75	8.15	8.46	8.45 ^{a}	54.90 ^{c}	53.03 ^{e}	52.80 ^{e}	53.58	30.87	32.00	31.93	31.60 ^{a}	14.63 ^{a}	9.75 ^{bc}	11.03 ^{b}	11.80
PD3	39.45	38.93	38.12	38.83 ^{a}	9.56	8.52	8.23	8.77 ^{a}	55.47 ^{b}	54.57 ^{c}	55.07 ^{bc}	55.04	30.43	31.30	31.00	30.91 ^{a}	8.28 ^{d}	9.66 ^{c}	9.03 ^{cd}	8.99
Mean	38.03 ^{a}	37.99 ^{a}	37.60 ^{a}		9.06 ^{a}	8.47 ^{ab}	8.36 ^{b}		55.55	53.76	53.73		30.74 ^{a}	31.48 ^{a}	31.45 ^{a}		10.48	9.34	11.12	
SEM		0.43				0.10				0.23				0.13				0.44		
p value																				
Planting density (PD)		0.019				0.289				<0.001				0.057				<0.001		
Soybean sowing time (ST)		0.231				0.003				<0.001				0.099				<0.001		
PD × ST		0.406				0.212				<0.001				0.610				<0.001		

Data are the mean and standard error of the mean of the samples. For items with significant interaction effects of PD × ST, significance analysis was conducted among different treatments. For items with insignificant interaction effects of PD × ST, significance analysis was conducted between the mean values of major factors and secondary factor. Lower case letters with different superscripts indicate the differences between different treatments ($p < 0.05$). SEM, standard error of the mean; PD1, the planting density of soybean was 120,000 plants ha^{-1}; PD2, the planting density of soybean was 150,000 plants ha^{-1}; PD3, the planting density of soybean was 180,000 plants ha^{-1}; ST1, the sowing time of soybean was 9 d before corn sowing; ST2, the sowing time of soybean was 0 d before corn sowing; ST3, the sowing time of soybean was 9 d after sowing corn; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC, water-soluble carbohydrate.

Table 5. Effects of sowing time and planting density of soybean on chemical composition of samples after 60 days of ensiling in corn-soybean strip intercropping system.

Item	DM (%)				CP (% of DM)				NDF (% of DM)				ADF (% of DM)				WSC (% of DM)			
	ST1	**ST2**	**ST3**	**Mean**	**ST1**	**ST2**	**ST3**	**Mean**	**ST1**	**ST2**	**ST3**	**Mean**	**ST1**	**ST2**	**ST3**	**Mean**	**ST1**	**ST2**	**ST3**	**Mean**
PD1	34.36	35.56	34.61	34.84 ^{b}	9.58	9.00	8.94	9.17 ^{b}	56.10	52.47	52.53	53.70 ^{a}	30.97	31.83	31.90	31.57 ^{a}	1.92 ^{c}	0.95 ^{d}	1.09 ^{d}	1.32
PD2	34.61	35.75	38.21	36.19 ^{ab}	9.67	9.48	9.52	9.56 ^{a}	52.67	54.23	52.40	53.10 ^{a}	31.90	31.77	32.63	32.10 ^{a}	3.09 ^{b}	4.34 ^{a}	4.90 ^{a}	4.11
PD3	38.54	36.28	36.34	37.05 ^{a}	9.58	9.98	9.71	9.76 ^{a}	51.43	52.03	52.60	52.02 ^{a}	33.03	32.00	31.40	32.14 ^{a}	1.34 ^{cd}	1.81 ^{c}	0.68 ^{d}	1.28
Mean	35.84 ^{a}	35.86 ^{a}	36.39 ^{a}		9.61 ^{a}	9.49 ^{a}	9.39 ^{a}		53.40 ^{a}	52.91 ^{a}	52.51 ^{a}		31.97 ^{a}	31.87 ^{a}	31.98 ^{a}		2.12	2.37	2.22	
SEM		0.40				0.10				0.40				0.20				0.30		
p value																				
Planting density (PD)		0.015				0.040				0.673				0.951				<0.001		
Sowing time (ST)		0.463				0.172				0.255				0.274				0.429		
PD × ST		0.120				0.125				0.190				0.070				<0.001		

Data are the mean and standard error of the mean of the samples. For items with significant interaction effects of PD × ST, significance analysis was conducted among different treatments. For items with insignificant interaction effects of PD × ST, significance analysis was conducted between the mean values of major factors and secondary factors. Lower case letters with different superscripts indicate the differences between different treatments ($p < 0.05$). SEM, standard error of the mean; PD1, the planting density of soybean was 120,000 plants ha^{-1}; PD2, the planting density of soybean was 150,000 plants ha^{-1}; PD3, the planting density of soybean was 180,000 plants ha^{-1}; ST1, the sowing time of soybean was 9 d before corn sowing; ST2, the sowing time of soybean was 0 d before corn sowing; ST3, the sowing time of soybean was 9 d after sowing corn; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC, water-soluble carbohydrate.

Table 6. Effects of sowing time and planting density of soybean on fermentation profile of samples after 60 days of silage in corn-soybean strip intercropping system.

Item	pH				NH_3-N/TN (%)				Lactic acid (mg g^{-1} DM)			
	ST1	ST2	ST3	Mean	ST1	ST2	ST3	Mean	ST1	ST2	ST3	Mean
PD1	3.82 ^{b}	3.83 ^{b}	3.87 ^{ab}	3.84	4.32	4.75	4.47	4.51 ^{a}	23.77 ^{b}	18.60 ^{c}	17.53 ^{c}	19.97
PD2	3.86 ^{ab}	3.85 ^{ab}	3.88 ^{a}	3.86	3.75	3.82	3.05	3.54 ^{b}	17.74 ^{c}	16.24 ^{cd}	31.38 ^{a}	21.79
PD3	3.87 ^{ab}	3.85 ^{ab}	3.83 ^{b}	3.85	3.50	3.04	4.00	3.51 ^{b}	21.02 ^{bc}	20.86 ^{bc}	13.50 ^{d}	18.46
Mean	3.85	3.84	3.86		3.86 ^{a}	3.87 ^{a}	3.84 ^{a}		20.84	18.57	20.80	
SEM			0.50				0.14				1.02	
p value												
Planting density (PD)			0.046				0.001				0.016	
Sowing time (ST)			0.232				0.995				0.064	
PD × ST			0.043				0.094				<0.001	

Item	Acetic acid (mg g^{-1} DM)				Propionic acid (mg g^{-1} DM)				Butyric acid (mg g^{-1} DM)			
	ST1	ST2	ST3	Mean	ST1	ST2	ST3	Mean	ST1	ST2	ST3	Mean
PD1	2.56 ^{ab}	2.28 ^{ab}	2.27 ^{ab}	2.37	3.75 ^{ab}	3.65 ^{ab}	3.63 ^{ab}	3.68	0.76 ^{ab}	0.80 ^{ab}	0.77 ^{ab}	0.78
PD2	2.01 ^{b}	2.05 ^{b}	2.75 ^{a}	2.27	3.89 ^{a}	3.31 ^{b}	3.09 ^{b}	3.43	0.79 ^{ab}	0.70 ^{b}	0.80 ^{ab}	0.76
PD3	1.99 ^{b}	2.80 ^{a}	2.41 ^{ab}	2.40	3.19 ^{b}	3.28 ^{b}	3.83 ^{a}	3.43	0.62 ^{b}	0.83 ^{a}	0.64 ^{b}	0.70
Mean	2.19	2.38	2.48		3.61	3.41	3.52		0.72	0.78	0.73	
SEM			0.08				0.07				0.02	
p value												
Planting density (PD)			0.250				0.291				0.267	
Sowing time (ST)			0.723				0.096				0.071	
PD × ST			0.030				0.004				0.011	

Data are the mean and standard error of the mean of the samples. For items with significant interaction effects of PD × ST, significance analysis was conducted among different treatments. For items with insignificant interaction effects of PD × ST, significance analysis was conducted between the mean values of major factors and secondary factors. Lower case letters with different superscripts indicate the differences between different treatments ($p < 0.05$). SEM, standard error of the mean; PD1, the planting density of soybean was 120,000 plants ha^{-1}; PD2, the planting density of soybean was 150,000 plants ha^{-1}; PD3, the planting density of soybean was 180,000 plants ha^{-1}; ST1, the sowing time of soybean was 9 d before corn sowing; ST2, the sowing time of soybean was 0 d before corn sowing; ST3, the sowing time of soybean was 9 d after sowing corn; NH3-N/TN, the ammonia-nitrogen content to total nitrogen.

3.4. Fermentation Profile of Corn and Soybean Mixed Silage

After 60 days of ensiling, different soybean planting densities and sowing times affect the fermentation quality of the corn and the soybean mixed silage (Table 6). The pH and the LA content were affected by planting density and the interaction of planting density with sowing time ($p < 0.05$). The LA content was averagely the highest at PD2ST3 (31.38 mg g^{-1} DM). The content of NH_3-N/TN was affected by planting density ($p < 0.05$) where there was a decrease in the increase in planting density, among which PD1 had the highest NH_3-N/TN. The content of AA, PA, and BA was affected by the interaction of planting density and sowing time ($p < 0.05$). The AA content of PD3ST2 was the highest (2.80 mg g^{-1} DM), followed by PD2ST3(2.75 mg g^{-1} DM). The PA content of PD2ST3 (3.09 mg g^{-1} DM) was lower than other treatments. The BA content of PD3ST2 was the highest (0.83 mg g^{-1} DM).

4. Discussion

4.1. Effects of Sowing Time and Density of Soybean on Yield and Chemical Composition

An intercropping system can effectively improve the utilization rate of multiple resources, thus increasing yields and land equivalent ratios. In the current study, the results showed that the total dry matter yield of corn and soybean in a strip intercropping system decreased with an increase in soybean planting density, which is similar to the results of Cardoso et al. [27] and Prasad and Brook [28], who reported that the variation of crop yield may be related to the difference of planting density, spatial distribution, and growth conditions. In the present study, to keep the corn planting density constant, we changed the corn plants spacing and adjusted the row spacing appropriately to increase the strip width to maintain the density of 60,000 plants ha^{-1} of corn among different treatments. Corn had an obvious edge growth effect after intercropping. This edge growth effect was due to the use of narrow strips grown with compact or semi-compact corn varieties which facilitated the edge growth effects of corn in any of the rows of the narrow strips; each row of corn could make the most of the resources of the wide row (soybean row), such as light radiation, etc. [38], and thus the decrease in proper plant spacing still maintained a crop yield at a comparable level. However, the increase in soybean planting density brought a decrease in the corn yield in the current study; plant spacing that is overly short usually leads to an intensification of intraspecies competition [19,34], and the edge growth effect may not be able to make up for the yield loss caused by intraspecies competition thus contributing to yield reduction [39]. Meanwhile, the spacing of the corn plants was only 12.83 cm at PD3 and the yield was significantly lower than that of the others. In the current study, the competition between species was observed as the corn yield decreased with the increase in soybean planting density, but the increase in soybean planting rows had increased with the increase in its planting density, which amplified the edge growth effects of corn to a certain extent and effectively alleviated the impact of yield reduction caused by the increase in corn plant spacing. Above all, proper control of intercropping density and spacing can effectively improve crop yield [40].

The current study mainly focused on the effects of different soybean planting densities and sowing times on the corn–soybean dry matter yield of a whole crop silage. The change in soybean sowing time had little effect on the total dry matter yield, which may be because the corn was the major yield, so the sowing time of soybean had little effect on the yield of corn.

Nitrogen in soybean is mainly distributed in pods and seeds [41]. In the current study, soybean sowing time had a significant effect on CP content. The soybean of ST1 was at the R8 stage (full maturity), the pods and the seeds had a higher proportion in the whole plant weight, so the CP content of ST1 was higher than others. The NDF content under treatment PD2 was significantly lower than that of other treatments, indicating that the appropriate planting density was conducive to reducing the NDF content [12,25]. At the same time, the NDF content decreased with the delay in sowing time when soybean planting density was PD1 or PD2. This may be because the earlier sowing time resulted in more mature soybean, plants making more use of environmental nutrients, light, etc., and the deposition of lignin, cellulose, and other structural carbohydrates in the plant's cell wall therefore increasing and creating a higher NDF content [42,43]. However, the NDF content of PD3 did not show such a trend, which may be due to the high planting density, as high planting density may exert some growth physiological impacts on plants. Moriri et al. [44] showed that with an increase in planting density, intraspecific competition intensified and the flowering and the maturity of plants were delayed, which may be the reason for the affected NDF content.

4.2. Effects of Sowing Time and Density of Soybean on Chemical Composition and Fermentation Profile after Ensiling

Silage that is well fermented usually has a higher nutrient content [45]. In the current study, the CP content increased with the increase in soybean planting density. Soybean can effectively make up for the low CP content of corn silage [9]. Baghdadi et al. [46] reported that the CP content also decreased significantly as the proportion of soybean in mixed silage decreased, and our study found similar results in that PD1ST3 had the lowest CP content. During ensiling, the WSC is the major substrate for microbial fermentation, so it usually decreases in a high quantity. In the current study, the WSC content of each treatment expectedly decreased, but PD2ST3 (4.90% of DM) and PD2ST2 (4.34% of DM) were higher than other treatments, which may be attributable to the different epiphytic microbes among the fresh matter of different treatments [9]. Guan et al. [47] reported that the number and the structure of epiphytic microbes in naturally fermented silage were also related to the WSC content, except for the chemical composition of the plant. Besides, the different sowing times and planting densities resulted in the differences in the microecological environment such as temperature and water variances, etc. [29,48,49], which then affected the plant growth along with their epiphytic microorganisms [50]. Meanwhile, our results showed that the PD2ST3 had the highest LA content, which may be due to the larger number of lactic acid bacteria in the fresh matter versus the others. The higher LA content could inhibit undesirable microbes fermenting WSC [51], and this may be the reason for its high WSC content.

The pH, NH_3-N/TN, and BA content of acceptable gramineous silage is usually lower than 4.2, 10%, and 1% of DM, respectively [45,52]. However, leguminous silage is considered acceptable with a pH below 4.5 and an NH_3-N/TN below 15%. In the present study, all treatments reached the standard of acceptable silage [45,50]. Jahanzad et al. [53] showed that an increase in soybean content would lead to an increase in the content of AA, BA, and PA in mixed silage. However, the different planting densities and sowing times of soybean may have affected the field micro-environment in terms of differences in light radiation, temperature, humidity, etc. [29,47,49], and such external factors changed the population and the structure of the epiphytic microbial flora [9], which then affected the fermentation product and the chemical composition, such as the organic acids and the DM content [54–56]. In the current study, the LA content of PD2ST3 was the highest, and it reduced pH and inhibited the growth of undesirable microbes more efficiently [51,57]. Therefore, the optimal choice was PD2ST3 from the perspective of chemical composition and fermentation quality in the conditions of the experiment. However, the microbial community of the fresh matter and the silage under different intercropping systems still require further study.

5. Conclusions

The total dry matter yield of corn–soybean intercropping decreased with an increase in soybean planting density, and the soybean at a planting density of 150,000 plants ha^{-1} and 9 days sowing later than corn (PD2ST3) had a relatively high CP, DM, and WSC and the lowest NDF content. After ensiling, the CP content increased with the increase in soybean planting density. The content of WSC in treatment PD2 was the highest among different soybean densities, and PD2ST3 had the highest LA content. The effects of soybean planting density on intercropping yield and silage quality were greater than sowing time, and the silage quality of PD2ST3 was better than the others. Therefore, the planting density of

150,000 plants ha^{-1} with a sowing time of 9 days later than corn for soybean was optimal for both silage quality and biomass yield in the conditions of the experiment.

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